# Abstract

Ribosomes are large ribonucleoprotein complexes, which perform all cellular protein synthesis. The biogenesis of a functional ribosome requires modifications, particularly ribosomal RNA modifications, which are a universal phenomenon that influence processes like ribosome assembly, rRNA maturation, translation fidelity and efficiency as well as antibiotic resistance. The most common rRNA modification is methylation, which is found on 24 nucleotides in *Escherichia coli* and clustered into functionally important regions of the ribosomes. Even though all the methylation sites and their responsible methyltransferases have been identified, their molecular function is in many instances still lacking. This is the case for the two methyltransferases, RlmB and RlmH, which are respectively responsible for the methylations leading to Gm2251 and $m^3\Psi$1915 of 23S rRNA in *Escherichia coli*. Both the RlmB and RlmH modifications are highly conserved and found in all three domains of life. However, previous studies have shown that their functions are, contrary to evolutionary "common sense", apparently dispensable. To seek an explanation to this paradox, the molecular function of these modifications was investigated through quantitative transcriptomics and proteomics studies. We found that in the absence of the *rlmB* and *rlmH* methyltransferase genes, transcripts and proteins related to flagellar biogenesis and motility as well as chemotaxis signaling pathways were highly expressed. This was further investigated by performing motility assay and transmission electron microscopy, which substantiated the Omics observations. The high expression of the flagella-related transcripts and proteins may be attributed to the key regulator LrhA, which was found down-regulated in both the Δ*rlmB* and Δ*rlmH* strain. LrhA is a transcriptional repressor that negatively controls the *flhDC* operon and thereby the expression of flagella and chemotaxis genes. The focus in this study was then placed on LrhA and how the RlmB and RlmH modifications affect the transcription of *lrhA*. Further investigations showed that the mRNA stability of *lrhA* was unaffected and no premature termination of the *lrhA* transcription was observed. However, a reduced β-galactosidase activity was observed for both the Δ*rlmB* and Δ*rlmH* strain, which can likely be attributed to transcriptional effects from the region upstream of the *lrhA* gene.

The results obtained in this PhD project are presented in Manuscript I and provides new insight into the effects of the RlmB and RlmH methyltransferases and their products Gm2251 and $m^3\Psi$1915, respectively, in *E. coli*.